

# Third Quarterly Report

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Project Title: Effect of Concentration and Temperature of Ethanol in Fuel Blends on Microbial and Stress Corrosion Cracking of High-Strength Steels

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For quarterly period ending: *March 15, 2009*

## Technical Status Section:

Technical efforts for the third quarter of the project period included development of mechanical testing capabilities and procedures, refinement of electrochemical testing capabilities and procedures, chemical and biological analysis of fuel grade ethanol collected from the ethanol industry infrastructure, and biological thin-film interfacial (metal substrate) studies.

### Ethanol Corrosion Literature Review

On-going literature review has continued, and an ethanol corrosion review paper is being drafted for submission to an archivable journal.

### Electrochemical Characterization of Statically Loaded U-bend Specimens in Ethanol

In an effort to expedite and complement the screening phase of the project, experiments have been designed to electrochemically characterize corrosive media solution variables (water, oxygen, and chloride concentrations) using u-bend specimens fabricated and loaded according to ASTM-G30. Solutions will be characterized using potentiodynamic polarization and impedance spectroscopy. pH will also be monitored with time.

Preliminary electrochemical measurements have been performed for an ASTM G-30 u-bend specimen in 200 proof ethanol using Cyclic Polarization with a scan rate of 1mV/s as seen in Figure 1. A modified Ag/AgCl electrode containing ethanol saturated with LiCl was prepared using a procedure developed by Brossia and Kelly<sup>\*</sup>. This electrode was used to minimize water pick-up during testing. Due to difficulties associated with maintaining a stable reference electrode potential, a new type of electrode is being fabricated for use.

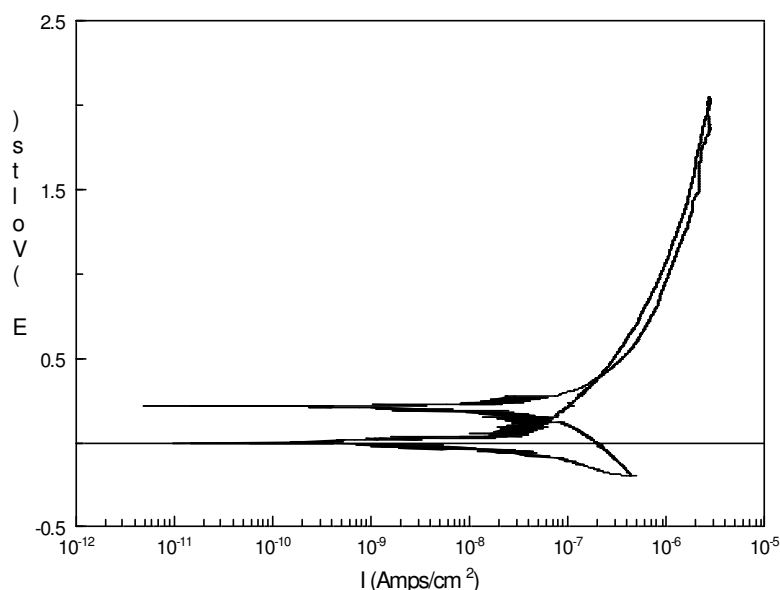


Figure 1: Cyclic polarization curve of ASTM A-36 u-bend specimens in Ethyl alcohol (200 proof, absolute ACS/UPS grade)

\*C.S. Brossia, R.G. Kelly, *Electrochimica Acta*, 41, 16, 1996, p. 2579-2585.

The manual ohmic compensation procedure was applied to the Polarization data by noting the impedance value at the highest frequency where the phase angle is close to zero in the EIS curve obtained after running the potentiodynamic scan. Corrosion parameters were calculated from the curve as shown in Table 1.

Table 1: Results obtained from the polarization analysis of Figure 1

Solution	Pure Ethanol
Cathodic Slope (mV/decade)	-159.3
Anodic Slope (mV/decade)	488.5
Corrosion Current Density ( $A/cm^2$ )	$3.256 \times 10^{-8}$
Corrosion Potential (V) (vs. Ag/AgCl/LiCl reference electrode)	-0.0186

### Cyclic Bent-Beam Testing in Ethanol

The design for the multi-specimen cyclic-loading bending fixture and bath assembly has been completed. All materials for the bath and fixture have been selected. Loading calculations have been performed to ensure that fixture strain will be within acceptable ranges for testing of tank and pipeline steels.

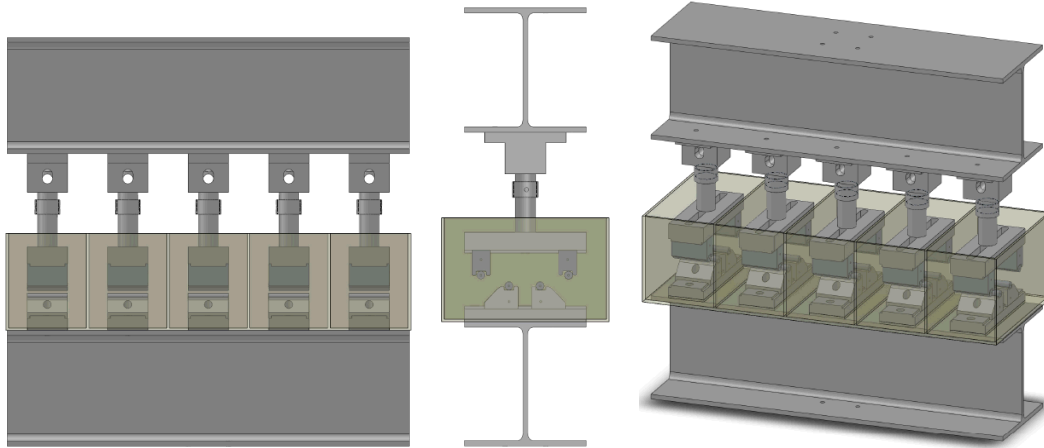


Figure 2: Front, side, and axonometric projections of the multi-specimen bending fixture assembly.

Fixture components will be fabricated out of ANSI 1018 mild carbon steel. All fixture components that may contact corrosive media will be coated to prevent degradation and remain insulated from the media. A high performance Novolac epoxy tank liner recommended for primary containment of FGE will be used. AkzoNobel has donated five gallons of Devchem 253 epoxy. The compartmented containment bath will be fabricated out of clear polyvinyl chloride (CPVC). Submerged fasteners will be fabricated out of Nylon 6/6. Coated fixture components, the bath interior and plastic fasteners will be pre-soaked in ethanol to remove any soluble residual polymer contaminants which could act as corrosion inhibitors. Two wide flange 6X6X15 beams will be used to maintain fixture rigidity. Adjustment collars with fixing screws will enable adjustment of the five individual loading systems to equilibrate differences in specimen vertical positioning introduced by machining variability. Potentiodynamic polarization and impedance spectroscopy will characterize corrosion behavior during cyclic loading.

## Field Contacts and Visits

Two ethanol production facilities have agreed to participate in the project. A third production facility has been contacted. Additional production facilities are being selected for participation. Efforts are being made to locate storage and distribution infrastructure with visible external microbiological growth. A representative from the Steel Tank Institute will be helping to facilitate this. Ethanol production facility personnel answered questions pertaining to ethanol-related corrosion issues. Samples will be collected from ethanol locations such as tank filters and other locations which may collect debris or harbor microbes at maintenance intervals. Ethanol-sludge samples from industry infrastructure will provide information about possible microbial presence and participation in material degradation.

## Microbiological Characterization of Field Fuel Grade Ethanol Samples

16S rRNA gene has been applied to a solids sample collected from the bottom of an ethanol storage tank. 16S rRNA gene sequencing determines the presence of microbial organisms in an environment by identifying their unique DNA sequences. While this technique can determine what microbes are present, it cannot determine if the microbes are metabolically active. Isolating DNA from the ethanol storage tank bottoms sample has proven difficult as the sample is high in iron and sulfur. A technique involving magnet separation and pre-cell lyses EDTA washes has given promising results. The extracted DNA has been subjected to 16S rRNA gene sequencing. The preliminary results are presented in Figure 2. Sequences were classified into a variety of bacterial divisions. The clone library was comprised predominately of sequences grouping with *Gammaproteobacteria* and *Firmicutes*. Sequences classified as *Gammaproteobacteria* included the presence of *Acinetobacter*, which have been shown to use ethanol as a carbon source. Sequences classified as *Firmicutes* included the presence of *Bacilli*. *Bacilli* are known spore formers and have been associated with biofilm formation as well as oxidation of iron and manganese. Spores are a mechanism employed by some microbes to survive harsh environments. Due to the difficulty of the DNA extraction procedure, the 16S rRNA gene sequencing results are currently being verified.

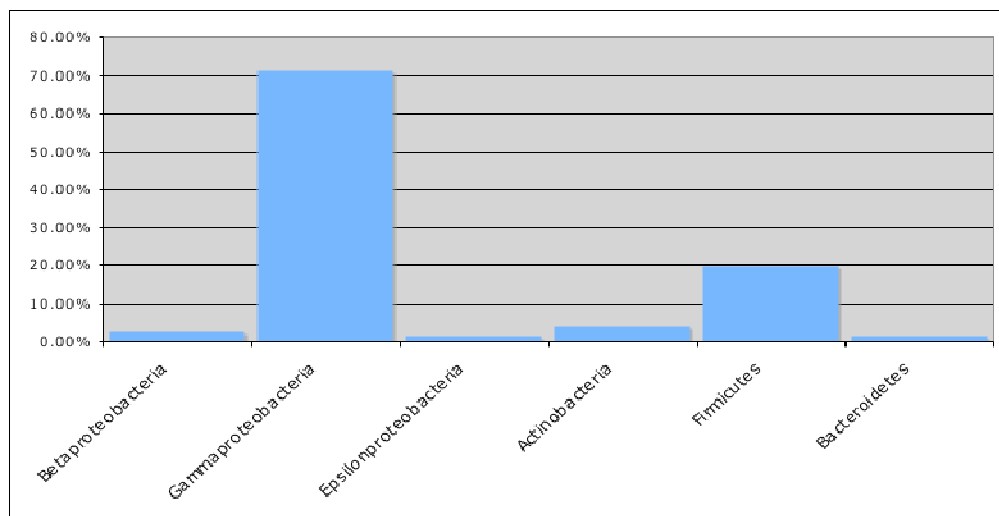


Figure 3: Results of 16S rRNA gene sequencing of an ethanol storage tank bottoms sample.

The chemical composition of an ethanol storage tank bottoms sample was evaluated with inductively coupled plasma optical emission spectroscopy (ICP-OES). The sample was high in iron and sulfur, which is consistent with reports in API Report 939D. The bottoms sample also contained significant amounts of aluminum, calcium, sodium, manganese, and potassium. The elevated levels of iron and manganese may suggest that the bottoms sample contained corrosion product from the steel tank.

#### Microbiological Iron Thin Film Studies in Ethanol

Experiments have been designed to grow microbes in a nanolayer of adsorbed water along a metal and ethanol solution interface. A very thin (20 nm) layer of iron has been deposited on glass microscope slides. A variety of methods, including light microscopy, will be used to examine the presence of microbial activity associated with possible water segregation along the Helmholtz double layer adhering to the metal surface. Variables to be studied are changes in water, fuel, metal cation, and anion concentrations in the ethanol. Initial tests have included only very low ethanol concentrations (approximately 1%), and have shown that biofilms can be grown on the thin layer of iron and that the biofilm is easily examined with microscopy as seen in Figure 4. Halos appearing around microbes that have colonized the iron-coated slide indicate that the microbes may be metabolizing iron on the slide.

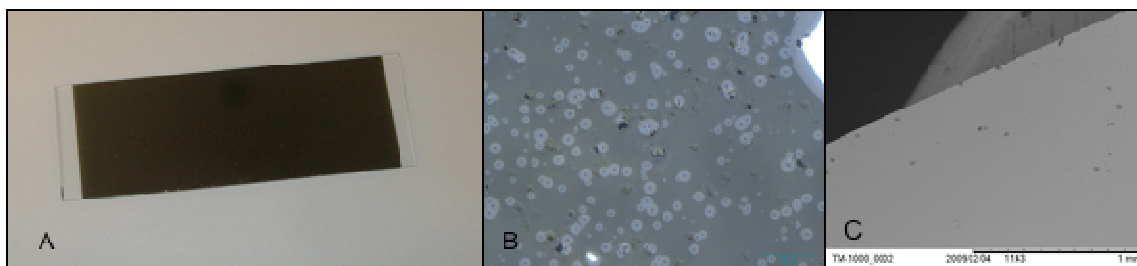


Figure 4: a) Microscope slide sputter-coated with a 20 nm iron layer. B) Microbial colonization on an iron-coated microscope slide c) Electron micrograph of an iron-coated slide

#### Biological Viability Studies in Ethanol

Efforts have been made to isolate viable microbes from a sample collected from the ethanol industry infrastructure. Some initial microbial growth experiments have indicated that viable microbes may be present in the sample collected from an ethanol storage tank. These initial experiments have included seeding ASTM-A36 steel coupons placed in water-ethanol mixtures with the ethanol storage tank bottoms sample as well as other inoculums. Several different growth media containing varying concentrations of ethanol have also been inoculated with the ethanol storage tank bottoms sample. Microbial growth experiments will continue to explore possible environments in which microbes can survive and potentially contribute to microbiologically influenced corrosion.